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Phenolic Chemotaxonomy of the Genus *Pelea* A. Gray (Rutaceae)[1]

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ABSTRACT: The thin-layer chromatographic fingerprints of ether-soluble compounds, mostly phenolics, from methanolic extracts of nine species of the genus *Pelea* A. Gray are shown to be of potential use in chemotaxonomic classification of this genus. The phenolic compounds of *Pelea anisata* Mann are consistent between populations, and each of the eight other species tested have fingerprints that are unique.

THE GENUS *Pelea* A. Gray (Rutaceae) is found principally on the eight major Hawaiian Islands, except two species native to the Marquesas Islands. In Hawai'i, 70 species are now recognized, and the incidence of endemism is high (Stone 1969). *Pelea* includes few distinct species, and the numerous systematic works dealing with the genus have often been in conflict. Each species has characteristics that vary among individuals, and the pattern of variation of a given species may overlap with that of the next. Hence, morphological criteria alone have been insufficient for taxonomic clarification in some instances.

The odoriferous properties of *Pelea anisata* Mann have been attributed to phenolic constituents (Scheuer 1955), and the possible use of the essential oils as chemotaxonomic indicators has been investigated (Hudgins and Scheuer 1964).

The study reported here is a preliminary survey of the variations in phenolic constituents of species in the genus *Pelea*, and was undertaken to determine the possible use of thin-layer chromatographic fingerprinting in taxonomic work on this genus.

MATERIALS AND METHODS

Initially, one species, *Pelea anisata*, was analyzed to determine whether the phenolic thin-layer chromatograph (TLC) fingerprint was consistent in individuals from eight different populations. Secondly, the TLC fingerprints of eight other species were compared to determine whether each species has a unique phenolic fingerprint.

Fresh leaf material of *Pelea anisata* Mann was collected from eight populations in the Kōke'e region, Kaua'i, and each population was extracted separately. Leaves of *P. barbigera* (Gray) Hillebrand, *P. cinereops*, *P. clusidefolia* Gray, *P. elliptica* (Gray) Hbd. var. *elliptica* f. *coccinea* (St. John and Hume) Stone, *P. peduncularis*, *P. oahuensis*, *P. kaalaensis* St. John, and *P. rotundifolia* Gray were collected on O'ahu and Kaua'i. The location of each collection is listed in Table 1.

Five grams of fresh plant material were homogenized in 30 ml anhydrous methanol in a Waring blender and extracted for 24 hr at room temperature. The homogenate was filtered and extracted with light petroleum ether (bp 40–60° C) until free of chlorophylls and other pigments. An equal volume of water was added, and the methanol was evaporated in vacuo. The aqueous preparation was then extracted successively with 200 ml diethyl ether and 200 ml ethyl acetate. The ether fraction was evaporated to 50 ml and directly loaded on thin-layer plates of silica gel GF254 with a 250-μm thickness (Stahl 1965).

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TABLE 1

LOCATIONS OF COLLECTIONS OF *Pelea* LEAVES USED FOR EXTRACTION OF PHENOLIC COMPOUNDS

POPULATION	SPECIES	LOCATION
A	*P. anisata*	Alaka'i Swamp Trail, Kaua'i
B	*P. anisata*	Po'omau Canyon Trail, Kaua'i
C	*P. anisata*	Honopū Trail, Kaua'i
D	*P. anisata*	Kumuela Road, Kaua'i
E	*P. anisata*	Waininiua Trail, Kaua'i
F	*P. anisata*	Kumuela Road, Kaua'i
G	*P. anisata*	Kumuela Road, Kaua'i
H	*P. anisata*	Nualolo Trail, Kaua'i
	P. barbigera	Awa'awa'puhi Trail, Kaua'i
	P. oahuensis	Wilhelmina Rise Trail, O'ahu
	P. kaalaensis	Kōke'e, Kaua'i
	P. rotundifolia	Wilhelmina Rise Trail, O'ahu
	P. elliptica	Wilhelmina Rise Trail, O'ahu
	P. cinereops	Wilhelmina Rise Trail, O'ahu
	P. peduncularis	Kumuela Road, Kaua'i
	P. clusiaefolia	Alaka'i Swamp Trail, Kaua'i

NOTE: *Pelea anisata* population designations are identical to those in Figure 1.

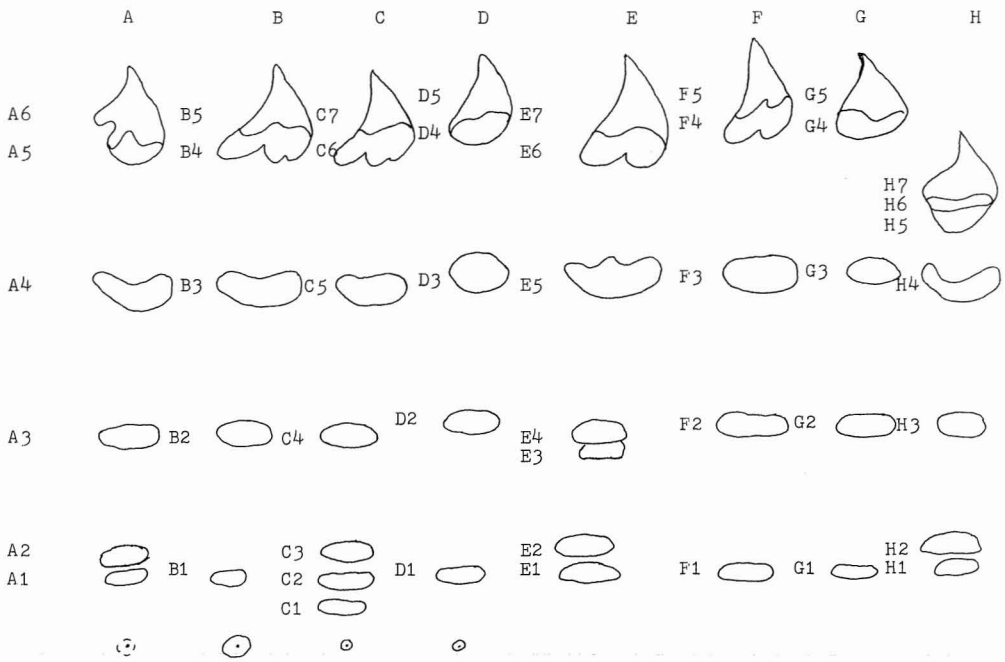

FIGURE 1. Outline fingerprints of compounds from the ether fraction of methanolic extracts of leaves from eight populations of *Pelea anisata*, as seen under long-wave ultraviolet light. Each fingerprint is from one population (labeled A-H), and compounds are numbered toward increasing Rf values.

Thin-layer chromatograms were developed in benzene : acetone 9 : 1, and visualized with the following phenolic reagents: ReA = diazotized *p*-nitroaniline (Smith 1960), ReB = benzidine reagent (Smith 1960), ReC = vanillin–HCl (Smith 1960), for catechin compounds, ReD = ferric chloride (Stahl 1965), ReE = sodium cobaltinitrite in acetic acid

TABLE 2

RADIOFREQUENCY (RF) VALUES AND COLOR REACTION TO PHENOLIC REAGENTS OF COMPOUNDS EXTRACTED FROM EIGHT POPULATIONS OF *P. anisata*

SPOT	Rf	LONG-WAVE UV	SHORT-WAVE UV	ReA	ReB	ReC	ReD	ReE	ReF	I/KI	UV ABSORPTION, λ MAX (nm)
A_1	0.125	(fl)pur	(fl)bl						bl		—
A_2	0.14	(fl)yel	absorb							or:re	—
A_3	0.25	(fl)pur	(fl)bl	white					bl	re:pur	—
A_4	0.49	(fl)bl	t = (fl)bl b = absorb	br	br				bl	or(b)	316
A_5	0.65		absorb	re	re:br	gr			pur	gr	260, 269, 285
A_6	0.8	(fl)bl	(fl)bl	yel	yel		gr	yel:br	bl	gr(f)	233
B_1	0.125	(fl)pur	(fl)bl						bl	gr	—
B_2	0.26	(fl)pur	(fl)bl	white					bl	re:pur	329
B_3	0.49	(fl)bl	t = (fl)bl b = absorb	br	br:re				bl	or(b)	317
B_4	0.65	(fl)bl	absorb	br:re	br:re	gr	pur	pur	pur	gr	259, 269, 285
B_5	0.82	(fl)bl	(fl)bl	yel	yel		gr	yel	bl	gr(f)	234
C_1	0.08	(fl)yelgr	—								—
C_2	0.125	(fl)bl	absorb						bl	gr	—
C_3	0.2	(fl)yelgr	absorb	pur	br			yel			—
C_4	0.3	(fl)yelgr	t = (fl)bl b = absorb	white	br				bl	re:pur	329
C_5	0.49	(fl)bl	absorb	br	gr:br				bl	or(b)	316
C_6	0.65	(fl)bl	absorb	br	re:br	br	pur		pur	or(b)	260, 269, 285
C_7	0.80	(fl)bl	(fl)bl	yel	yel	br	br	yel	bl	gr(f)	233
D_1	0.15	(fl)pur	—								—
D_2	0.34	(fl)bl	(fl)bl						bl		—
D_3	0.55	(fl)bl	(fl)bl	br	or				bl	or(b)	316
D_4	0.65	(fl)bl	absorb	re	or			br	pur	gr	259, 269, 285
D_5	0.79	(fl)bl	(fl)bl	yel	yel			br	bl	gr(f)	234

TABLE 2 (*Cont.*)

RADIOFREQUENCY (RF) VALUES AND COLOR REACTION TO PHENOLIC REAGENTS OF COMPOUNDS EXTRACTED FROM EIGHT POPULATIONS OF P. *anisata*

SPOT	Rf	LONG-WAVE UV	SHORT-WAVE UV	ReA	ReB	ReC	ReD	ReE	ReF	I/KI	UV ABSORPTION, λ MAX (nm)
E_1	0.125	(fl)pur	(fl)bl								—
E_2	0.15	(fl)yel	absorb	pink					bl	or	—
E_3	0.23	(fl)yel	absorb								—
E_4	0.3	(fl)pur	(fl)bl	white	br				bl	re:pur	331
E_5	0.49	(fl)bl	t = (fl)bl b = absorb	br	br				bl	or(b)	314
E_6	0.65	(fl)bl	absorb	br	re:br	gr	pur	pur	pur	gr	259, 270, 286
E_7	0.78	(fl)bl	(fl)bl	yel	yel		gr	br	bl	gr(f)	233
F_1	0.12	(fl)pur									—
F_2	0.34	(fl)bl	(fl)bl						bl		—
F_3	0.5	(fl)bl	(fl)bl	br	or					or	316
F_4	0.74	(fl)bl	absorb	br	or				pur	gr	258, 270, 286
F_5	0.85	(fl)bl	(fl)bl	yel	yel				bl	gr(f)	232
G_1	0.12	(fl)bl	—								—
G_2	0.3	(fl)bl	—	white					bl		—
G_3	0.48	(fl)bl	(fl)bl	br	or				bl	or	316
G_4	0.75	(fl)bl	absorb	pink	br				bl	gr	260, 271, 285
G_5	0.85	(fl)bl	(fl)bl	br	yel		pur	yel:br	bl	gr(f)	233
H_1	0.11	(fl)pur	(fl)pur						bl		—
H_2	0.15	(fl)yel	absorb	pur	or						—
H_3	0.27	(fl)pur	(fl)bl	white					bl		329
H_4	0.45	(fl)bl	(fl)bl	br	br				bl	or	314
H_5	0.55	(fl)bl	absorb	yel	pink				bl		234, 256
H_6	0.65	(fl)bl	absorb	br	br	gr	pur		pur	gr	260, 269, 285
H_7	0.83	(fl)bl	(fl)bl	yel	yel		gr			gr	234

NOTE: (fl) = fluorescent, (b) = bright, (f) = faint, t = top, b = bottom, ReA = *p*-nitroaniline, ReB = Benedicts, ReC = vanillin-HCl, ReD = ferric chloride, ReE = $NaCo(NO_3)_3$, ReF = Folin-Ciocalteau, br = brown, yel = yellow, pur = purple, gr = green, bl = blue, re = red, or = orange.

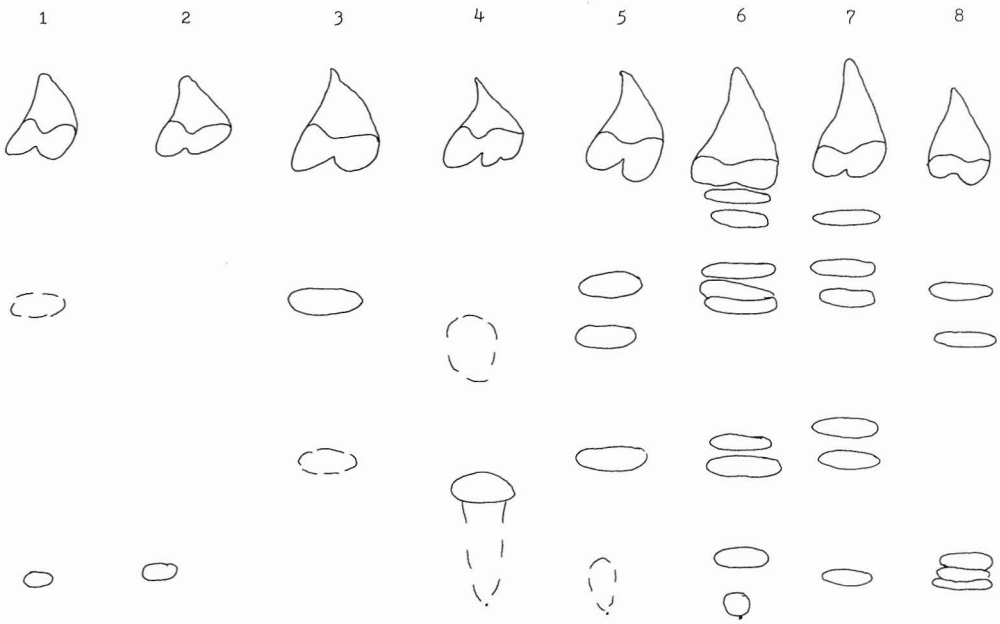

FIGURE 2. Outline fingerprints of compounds found in the ether-soluble fraction of methanol extracts from eight species of *Pelea*: 1 = *P. barbigera*, 2 = *P. kaalaensis*, 3 = *P. oahuensis*, 4 = *P. rotundifolia*, 5 = *P. elliptica*, 6 = *P. cinereops*, 7 = *P. peduncularis*, 8 = *P. clusiaefolia*.

(Bhatia et al. 1971), and ReF = Folin-Ciocalteau (Stahl 1965). *Pelea anisata* extracts were also tested with 0.5 percent I_2/KI (Abdel-Hay et al. 1965), which reacts red-purple in the presence of coumarin compounds. Finally, the spots were scraped from the plates and eluted with methanol, centrifuged, and the ultraviolet (UV) spectra determined with a Beckman ACTA III UV spectrophotometer (Spencer and Beggs 1966). No attempt was made at this time to identify the phenolics present, because visualization of overall fingerprints was the major objective. However, the general class of phenolic compounds to which a spot belongs can be deduced from the reaction to these reagents.

RESULTS

Figure 1 outlines the phenolic compounds extracted from eight populations of *Pelea anisata*, as visualized under long-wave UV light. The reaction of these compounds to the reagents and the wavelength of maximum absorption (λ max) of the UV spectra are summarized in Table 2. First, all *P. anisata* populations have in common five compounds, four of which are phenolic. The first ($A_1 = B_1 = C_2 = D_1 = E_1 = F_1 = G_1 = H_1$) has an Rf = 0.12 and is not phenolic, as shown by its lack of absorbency in the UV range. The second ($A_3 = B_2 = C_4 = D_2 = E_4 = F_2 = G_2 = H_3$), third ($A_4 = B_3 = C_5 = D_3 = E_5 = F_3 = G_3 = H_4$), fourth ($A_5 = B_4 = C_6 = D_4 = E_6 = F_4 = G_4 = H_5$), and fifth ($A_6 = B_5 = C_7 = D_5 = E_7 = F_5 = G_5$) are phenolic in nature, as shown by their reactions to phenolic reagents and their UV spectra. Note that spots A_3 and D_2 did not absorb in the UV range, however, this is likely due to the extremely small concentrations in which they were recovered from the chromatograms. They do show the proper reaction to phenolic reagents, and hence are assumed to be phenolic constituents. The fourth compound is of catechin structure, as indicated by its reaction with vanillin–HCl, and the third compound is probably a coumarin derivative (Table 2).

Second, variations in the fingerprints between populations exist, notably at Rf = 0.15

TABLE 3

REACTION TO PHENOLIC REAGENTS ON TLC PLATES OF COMPOUNDS EXTRACTED FROM EIGHT SPECIES OF *Pelea* (LISTED BY SUBSECTION OF THE GENUS)

SPECIES	SPOT	Rf	LONG-WAVE UV	SHORT-WAVE UV	ReA	ReB	ReC	ReD	ReE	ReF
Apocarpae										
P. barbigera	1	0.09	(fl) yel				pink			
	2	0.38	(fl) pur			br				
	3	0.65	(fl) bl	absorb	br	re		pur	yel	bl:pur
	4	0.79	(fl) bl	(fl) bl	br	yel	pink	pur	yel	bl
P. cinereops	1	0.09	(fl) pink			or	pink			
	2	0.15	(fl) bl	absorb	yel	br	pink			
	3	0.20	(fl) re				pink			
	4	0.24	(fl) bl			yel	pur			
	5	0.34	(fl) bl						yel	bl
	6	0.36	(fl) re							
	7	0.40	(fl) bl	absorb	yel	or:br				
	8	0.51	(fl) bl		yel	re	pur			
	9	0.62	(fl) re(b)		pink:pur	gr	pur			gr
	10	0.66	(fl) bl	absorb		yel	gr			bl
	11	0.72	(fl) bl	(fl) bl		yel	gr			bl
P. elliptica	1	0.09	(fl) yel	absorb		yel(b)	pink			
	2	0.21	(fl) pur	absorb						
	3	0.30	(fl) pur							
	4	0.38	(fl) bl							bl
	5	0.68	(fl) bl	absorb	br	br	gr	pur	br	bl:gr
	6	0.79	(fl) bl	(fl) bl	br	yel	pink	yel	yel	bl
Megacarpae										
P. kaalaensis	1	0.08	(fl) yel		br					
	2	0.65	(fl) bl	absorb	br	br	pink	pur	br	bl:gr
	3	0.77	(fl) bl	(fl) bl	br	yel		pur	yel	bl
P. rotundifolia	1	0.12	(fl) yel	absorb			pink			
	2	0.29	(fl) yel		br					
	3	0.65	(fl) bl	absorb	br	br	gr	pur	br	bl:gr
	4	0.72	(fl) bl	(fl) bl	br	yel		yel	yel	bl

TABLE 3 (*Cont.*)

SPECIES	SPOT	Rf	LONG-WAVE UV	SHORT-WAVE UV	ReA	ReB	ReC	ReD	ReE	ReF
Cubicarpae										
P. oahuensis	1	0.19	(fl) pur	absorb			pink			
	2	0.38	(fl) pur	(fl) bl						
	3	0.68	(fl) bl	absorb	br	re:br	gr	gr	br	bl:gr
	4	0.80	(fl) bl	(fl) bl	br	yel	pink		yel	bl
P. peduncularis	1	0.12	(fl) pur	absorb	pink	or	pink:pur			
	2	0.23	(fl) bl	absorb	pink	or:re	pink:pur			pur
	3	0.26	(fl) yel	absorb		pur	pink			pur:bl
	4	0.36	(fl) yel	absorb	br	pink	pur		yel	
	5	0.42			yel	yel				
	6	0.53			pink(b)	br				
	7	0.65	(fl) bl	absorb	pur	re	gr		re	pur
	8	0.72	(fl) bl	(fl) bl	yel	br	gr		yel	bl:gr
Pelea										
P. clusiaefolia	1	0.14	(fl) bl		yel	br	pink			
	2	0.15					pink			
	3	0.16					pink			
	4	0.31	(fl) bl		yel	yel	pink			bl
	5	0.37			yel	re	pur			bl
	6	0.63	(fl) bl	absorb	pink	re	bl:gr		re	pur
	7	0.74	(fl) bl	(fl) bl	yel	br	gr		yel	gr:bl

NOTE: See Table 2 for key to abbreviations.

in populations A, E, and H. However, these compounds do not exhibit characteristic aromatic UV absorption, and hence are not phenolic. The four phenolic constituents are very similar among spatially distinct populations of this species.

The TLC of eight other species of *Pelea* are outlined in Figure 2, and the reactions to reagents are summarized in Table 3. The UV spectral analysis was not performed; thus, it cannot be determined whether these compounds are phenolic. Each species extracted thus far has two compounds in common (those with the two highest Rf values). However, each species clearly has a unique overall fingerprint. Note in particular *Pelea oahuensis* and *P. peduncularis*; although morphologically similar, they are chemically distinct.

DISCUSSION

The consistency of the phenolics within populations of one species and the variability of phenolic patterns between species indicate that comparison of phenolic components should aid further clarification of the genus *Pelea*. However, determining the usefulness of this method requires the extraction of other *Pelea* species and the identification of the phenolics extracted. The materials for several of the species tested were from herbarium specimens. These were compared with extractions from fresh material of the same species and collection, and the phenolic constitution was nearly identical. The method described may also be useful for identification by means of chemical comparison with previously identified herbarium specimens.

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